(iii) plating the dissociated cells in feeder-independent culture on a substratum and in a media comprising fibroblast growth factor and chick embryo extract; and

(b) inducing the isolated, pure, homogeneous population of neuroepithelial stem cells to differentiate in vitro by replating the isolated, pure, homogeneous population of neuroepithelial stem cells onto a fibronectin substrate and in a media comprising chick embryo extract, NGF, FGF and EGF, thereby generating said neural crest stem cells.--

REMARKS

Claims 1-13 are pending in the instant application. Claims 1-13 have been rejected. Claim 1 and 6 have been amended. Claim 7 has been canceled in light of the amendment to claim 6. New claim 15 has been added. No new matter has been added by these amendments. Reconsideration is respectfully requested in light of these amendments and the following remarks.

I. Objection to Claim 1

Claim 1 has been objected to as the Examiner suggests that the term "homogenous" should be changed to "homogeneous". Therefore,

in an earnest effort to advance the prosecution of this case, Applicants have amended claim 1 per the Examiner's suggestion. Withdrawal of this objection is therefore respectfully requested.

II. Rejection of Claims 1-13 under 35 U.S.C. § 112, first paragraph

Claims 1-13 have been rejected under 35 U.S.C. § 112, first paragraph, as the Examiner suggests that the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to practice the invention commensurate in scope with these claims.

The Examiner has acknowledged the specification to be enabling for a method of producing neural crest stem cells from a population of rat neuroepithelial stem cells wherein the neuroepithelial cells are induced to differentiate into neural crest stem cells *in vitro* by replating the neuroepithelial stem cells onto fibronectin substrate and in media comprising CEE, NGF, FGF, EGF and optionally BMP-2. Therefore, in an earnest effort to advance the prosecution, Applicants have added new claim 15 which is drawn to the subject matter which the Examiner has acknowledged to be enabled.

However, Applicants respectfully disagree with the Examiner's suggestion that the specification is not enabling for other mammalian species.

Various prior art teachings are indicative of neural crest development occurring at equivalent stages of development in various species including, but not limited to, rat, mouse, chick, zebrafish and human. Examples of such references include that of Stemple and Anderson (Dev. Biol. 1993 159(1) 12-23 and Cell 1992 71(6):973-85). More recently published review articles also are indicative of neural crest development occurring at equivalent stages of development. Examples include Shakil et al. Hybridoma 2001 20(3):199-203; Selleck and Bronner-Fraser Int. J. Dev. Neurosci. 2000 18(7):621-7; Dorsky et al. Bioessays 2000 22(8):708-16; Raible and Kruse J. Comp. Neural. 2000 29;421(2):189-98; Shastry et al. Int. J. Neurosci. 2001 108(1-2):109-26; and Lakkis and Tennekoon J. Neurosci Res. 2000 62(6):755-63). Copies of any of these references can be provided to the Examiner upon request. Clearly, the teachings of the instant application relating to the rat could be routinely adapted to other mammalian species based upon what was known at the time of filing this application about the equivalent developmental stages in that particular species. For example, for mouse the equivalent stage of development is known

to be E8.5, for chick and quail it is somite stage 13-20, and for humans it is embryonic week 5 to 8.

The test of enablement, as set forth MPEP § 2164.01, is whether one reasonably skilled in the art could make or use the invention from the disclosure in the patent coupled with information known in the art without undue experimentation. The instant application demonstrates for the first time a method for generating neural crest stem cells from neuroepithelial stem cells in a mammalian species. The exemplary mammalian species used was the rat. However, from the teachings provided in the instant application coupled with information known regarding equivalent developmental stages in other mammalian species, one of skill in the art could routinely substitute neuroepithelial stem cells from other mammalian species at the equivalent developmental stage to generate neural crest stem cells for that species. Such substitution clearly does not constitute undue experimentation and is enabled by the teachings of the instant application.

Applicants also respectfully disagree with the Examiner's reasoning behind the suggestion that the specification is not enabling for inducing neuroepithelial stem cells to differentiate into neural crest stem cells by the claimed method because steps in the method result in differentiation of neuroepithelial stem cells

to cells beyond the neural crest stem cell. The Examiner relies upon Examples 5-7 of the instant application to support this suggestion. However, the NEP cells in Examples 5-7 were not treated by the same steps of the claimed method. In Examples 5-7, the cells were not plated in media comprising FGF and CEE as required in step (iii) of claim 1. Accordingly, these Examples are not relevant to the instant claimed invention and provide no reason to doubt the objective truth of statements in the specification that the method as claimed will generate neural crest stem cells.

It is therefore respectfully requested that this rejection under 35 U.S.C. § 112, first paragraph be withdrawn.

III. Rejection of Claims 6-7 under 35 U.S.C. § 112, second paragraph

Claims 6 and 7 have been rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Specifically, the Examiner suggests that claim 6 is rendered vague and indefinite by the phrase "wherein said inducing comprises withdrawing a mitogen" because it is unclear if the mitogen is FGF, as recited in amended claim 1, or a

different mitogen. Accordingly, in an earnest effort to advance the prosecution of this case, Applicants have amended claim 6 to clarify that the mitogen is FGF. Claim 7 has been canceled in light of the amendment to claim 6.

Withdrawal of this rejection under 35 U.S.C. § 112, second paragraph, is respectfully requested in light of these amendments.

IV. Conclusion

Applicants believe that the foregoing comprises a full and complete response to the Office Action of record. Accordingly, favorable reconsideration and subsequent allowance of the pending claims is earnestly solicited.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The

attached page is captioned "Version with Markings to Show Changes Made."

Respectfully submitted,

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Date: **July 25, 2001**

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the claims:

Claim 7 has been canceled.

The claims have been amended as follows:

1. (amended) A method for generating mammalian neural crest stem cells comprising:

(a) isolating a pure, homogeneous population of mammalian neuroepithelial stem cells derived from the neural tube from a mammalian embryo at a stage of embryonic development after closure of the neural tube by:

(i) removing a sample of neural tube tissue from a mammal at a stage of embryonic development after closure of the neural tube;

(ii) dissociating cells comprising the sample of neural tube tissue removed from the mammal; and

(iii) plating the dissociated cells in feeder-independent culture on a substratum and in a media comprising fibroblast growth factor and chick embryo extract; and

(b) inducing the isolated, pure, ~~homogenous~~ homogeneous population of neuroepithelial stem cells to differentiate in vitro

by replating the isolated, pure, ~~homogenous~~ homogeneous population of neuroepithelial stem cells on laminin-coated substrate, withdrawing fibroblast growth factor or chick embryo extract from the isolated, pure, ~~homogenous~~ homogeneous population of neuroepithelial stem cells, or adding a dorsalizing agent to the isolated, pure, ~~homogenous~~ homogeneous population of neuroepithelial stem cells, thereby generating said neural crest stem cells.

6. (amended) The method of claim 1 wherein said inducing comprising withdrawing ~~a mitogen~~ FGF.